Arsenic in Urine and Hair by ICP/MS

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1 Introduction

This procedure is used to detect and quantitate arsenic (As) in urine and hair specimens.

2 Scope

Analyses	☒ Screening ☒ Confirmation ☒ Quantitation
Matrices	Urine, hair
Analytes	Arsenic (unspeciated)
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 Principle

Urine specimens are mixed with the Indium Internal Standard Working Solution, diluted up to 10 mL in 2% Nitric Acid in Water Solution and analyzed contemporaneously with a complete matrix matched calibration curve and duplicate sets of matrix matched controls. Hair specimens are weighed, completely digested in tetramethylammonium hydroxide (TMAH), mixed with the Indium Internal Standard Working Solution, diluted up to 10 mL in 2% Nitric Acid in Water Solution and analyzed contemporaneously with a complete urine calibration curve, duplicate sets of controls in urine, a Negative Control Hair sample and duplicate sets of Positive Control Hair samples.

4 Specimen Criteria

This procedure is validated for urine and hair. Typically for urine, two 100 µL samples are analyzed. For hair, two 5.5 mg samples are analyzed. However, if it is indicated that the arsenic concentration is above the procedure's linear range, smaller sample sizes or further dilutions of the samples may be analyzed.

5 Equipment

5.1 Equipment

10 mL and 25 mL volumetric flasks, class A, PMP (VITLAB® or equivalent)
100 mL volumetric flask, class B, PP (Nalgene® or equivalent)
1 L volumetric flask, class B, PP (Nalgene® or equivalent)
25 mL plastic graduated cylinder (Nalgene® or equivalent)
Balance capable of measuring ± 0.1 mg

Routine laboratory supplies including but not limited to: pipettes, disposable pipettes, forceps, hand shears, etc.

Vortexer

5.2 Consumables

15 mL and 50 mL conical tubes with screw tops, PP (Falcon® or equivalent)

5.3 Instruments

A. Inductively Coupled Plasma-Mass Spectrometer with a collision cell installed (Thermo-Fisher iCAP Q or equivalent)
B. Autosampler (CETAC ASX-260 or equivalent)

5.4 Software

Component	Software	Version
Operating System	Microsoft Windows	7 Pro SP 1
Mass Spectrometer	Chromeleon	7.2 SR5
	Qtegra ISDS	2.10 SR1

5.5 Chemicals/Reagents

5.5.1 Purchased

Concentrated nitric acid (Optima grade)

Water (Deionized, 18 MΩ)

Tetramethylammonium hydroxide (TMAH), 25% w/w aqueous solution (Electronic grade)

Methanol (HPLC grade or better)

5.5.2 Prepared

2% Nitric Acid in Water Solution (v:v)

To a 1 L Nalgene® volumetric flask, add approximately 800 mL of deionized water. Add 20 mL of Optima grade concentrated nitric acid, fill to the mark with deionized water and mix well. Store at room temperature in plastic. Stable for at least one year.

5.6 Standards/Controls

Stability and storage determined by manufacturer unless otherwise noted.

5.6.1 Purchased

Indium Internal Standard Stock Solution (1 mg/L in 2% nitric acid solution)

Purchased from SPEX CertiPrep or an equivalent supplier.
Arsenic Calibrator Stock Solution (1 mg/L in 2% Nitric Acid in Water Solution)
Purchased from SPEX CertiPrep or an equivalent supplier.
Arsenic Control Stock Solution (100 mg/L in 2% nitric acid solution)
Purchased from High Purity Standards or an equivalent supplier.

5.6.2 Prepared

5.6.2.1 Internal Standard

Indium Internal Standard Working Solution (10 µg/L in 2% Nitric Acid in Water Solution)
To a 100 mL Nalgene® volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 80 mL of 2% Nitric Acid in Water Solution. Add 1 mL of Indium Internal Standard Stock Solution, fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.

5.6.2.2 Calibration

Arsenic Calibrator Working Solutions for Urine (10 µg/L – 1000 µg/L)
The following table shows the preparation of the individual Calibrator Working Solutions for Urine. The Calibrator Working Solutions are prepared in individual 25 mL class A volumetric flasks that have been washed with 2% Nitric Acid in Water Solution. Store at room temperature in plastic. Fill to the mark with 2% Nitric Acid in Water Solution and mix well. Stable for at least one year.

5.6.2.2.1 Calibration Scheme

Calibrator Working Solutions for Urine (µg/L)	Volume of Arsenic Calibrator Stock Solution (mL)
10	0.250
25	0.625
50	1.25
100	2.5
250	6.25
500	12.5
800	20.0
1000	25.0

5.6.2.3 Control

Arsenic Intermediate Control Working Solution (10 mg/L in 2% Nitric Acid in Water Solution)
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 2.5 mL of the Arsenic Control Stock Solution (100 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
Arsenic High Control Working Solution for Urine (800 µg/L in 2% Nitric Acid in Water Solution)
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 2.0 mL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
Arsenic Low Control Working Solution for Urine (30 µg/L in 2% Nitric Acid in Water Solution)
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 75 µL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
Arsenic High Control Working Solution for Hair (500 µg/L in 2% Nitric Acid in Water Solution)
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 1.25 mL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
Arsenic Low Control Working Solution for Hair (50 µg/L in 2% Nitric Acid in Water Solution)
To a 25 mL class A volumetric flask that has been washed with 2% Nitric Acid in Water Solution, add about 15 mL of 2% Nitric Acid in Water Solution. Add 125 µL of the Arsenic Intermediate Control Working Solution (10 mg/L), fill to the mark with 2% Nitric Acid in Water Solution and mix well. Store at room temperature in plastic. Stable for at least one year.
Negative Control Urine
Prepared from in-house anonymous donations that are pooled. Collected negative specimens are screened for arsenic. If arsenic is present in an individual specimen, the level must be below 10 µg/L. Combine and stored refrigerated in plastic. Stable for at least one year.
Negative Control Hair
Prepared from in-house anonymous donations. Collected negative specimens are screened for arsenic. If arsenic is present, the level must be below 1 ng/mg (50 µg/L). Store at room temperature in paper. Stable indefinitely.

6 PROCEDURE

6.1 Urine Analysis

	Calibration		Note/Reference
	A. To individual 10 mL Vitlab® volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:		
☐	1. Approximately 5 mL 2% Nitric Acid in Water Solution		
☐	2. 100 µL pooled in-house Negative Control Urine		
	3. 100 µL of the appropriate Calibrator Solution (µg/L)		
☐	i. Cal 0 (deionized water)		
☐	ii. Cal 10		
☐	iii. Cal 25		
☐	iv. Cal 50		
☐	v. Cal 100		
☐	vi. Cal 250		
☐	vii. Cal 500		
☐	viii. Cal 800		
☐	ix. Cal 1000		
☐	4. 100 µL Indium Internal Standard Working Solution		
☐	5. QS with 2% Nitric Acid in Water Solution mix well		
☐	B. Transfer calibrators to labeled 15 mL Falcon® tubes		
	Control (prepared in duplicate for quantitative analysis)		
	C. To individual 10 mL Vitlab® volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:		
☐	1. Approximately 5 mL 2% Nitric Acid in Water Solution		
☐	2. 100 µL pooled in-house Negative Control Urine		
	3. 100 µL of the appropriate Control Solution (µg/L);		
☐	i. Control 30 – Low Control W/S		
☐	ii. Control 800 – High Control W/S		
☐	4. 100 µL Indium Internal Standard Working Solution;		
☐	5. QS with 2% Nitric Acid in Water Solution mix well;		
☐	D. Transfer controls to labeled 15 mL Falcon® tubes.		

	Unknowns (prepared in duplicate for quantitative analysis)		
	E. To individual 10 mL Vitlab® volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:		
☐	1. Approximately 5 mL 2% Nitric Acid in Water Solution		
☐	2. 100 µL of unknown sample urine		
☐	3. 100 µL of deionized water		
☐	4. 100 µL Indium Internal Standard Working Solution;		
☐	5. QS with 2% Nitric Acid in Water Solution mix well;		
☐	F. Transfer samples to labeled 15 mL Falcon® tubes.		

6.2 Hair Analysis

Controls

	A. To individually labeled 15 mL Falcon® tubes:		
☐	1. Add a minimum of 27.5 mg of negative hair.		
☐	2. Accurately record the mass to the nearest 0.1 mg.		
☐	3. Based upon the recorded mass, add enough TMAH to establish a solution of 5 mg of hair per 100 uL of TMAH. (For example, 550 µL TMAH is needed for 27.5 mg of hair.)		
☐	4. Allow the hair to completely digest, vortexing occasionally. (This process typically takes at least 8 hours, and the process may be left to proceed overnight.)		
	B. To individual 10 mL Vitlab® volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:		
☐	1. Approximately 5 mL 2% Nitric Acid in Water Solution		
☐	2. 100 µL of negative hair digest (step A, above)		
	3. 100 µL of appropriate Control Solution (ng/mg)		
☐	i. Negative – Deionized Water		
☐	ii. Control 1 – Low Control Hair W/S (duplicate)		
☐	iii. Control 10 – High Control Hair W/S (duplicate)		
☐	4. 100 µL Indium Internal Standard Working Solution;		
☐	5. QS with 2% Nitric Acid in Water Solution mix well;		
☐	C. Centrifuge samples at 10,000 rpm x 5 minutes,		

☐	D. Transfer samples to new labeled 15 mL Falcon® tubes.		
	Unknowns		
	E. To individually labeled 15 mL Falcon® tubes:		
☐	1. Add a minimum of 5.5 mg of specimen hair in duplicate		
☐	2. Accurately record the mass to the nearest 0.1 mg.		
	Note: 5.5 mg is a small amount of hair. In order to ensure that a representative hair sample is analyzed, a larger amount of hair may be cut into small snippets and mixed before removing the 5.5 mg sample. Alternatively, a larger hair sample may be cryoground to mix it well.		
☐	3. Based upon the recorded mass, add enough TMAH to establish a solution of 5 mg of hair per 100 uL of TMAH. (For example, 110 µL TMAH is needed for 5.5 mg of hair.)		
☐	4. Allow the hair to completely digest, vortexing occasionally. (This process typically takes at least 8 hours, and the process may be left to proceed overnight.)		
	F. To individual 10 mL Vitlab® volumetric flasks that have been washed with 2% Nitric Acid in Water Solution add:		
☐	1. Approximately 5 mL 2% Nitric Acid in Water Solution		
☐	2. 100 µL of specimen hair digest (step D, above)		
☐	3. 100 µL of deionized water		
☐	4. 100 µL Indium Internal Standard Working Solution;		
☐	5. QS with 2% Nitric Acid in Water Solution mix well;		
☐	G. Centrifuge samples at 10,000 rpm x 5 minutes.		
☐	H. Transfer samples to new labeled 15 mL Falcon® tubes.		

6.3 Typical Instrument Sequence

Analyze calibration samples, control samples and unknown samples by ICP/MS using the instrumental conditions in Section 9 below.

Note: A Negative Control Urine (BLK) should be first in the sequence, followed by the calibrators (STDs), and unknown samples (UNKNOWN) bracketed by Positive Control Urines (QC – LCS). The Negative Control Urine may be reanalyzed as an UNKNOWN between specimens. When hair samples are analyzed, the Negative Control Hair should be analyzed after the urine specimens (as an UNKNOWN) followed by unknown samples (UNKNOWN) bracketed by Positive Control Hair samples (QC – LCS).

7 ANALYTICAL PARAMETERS

The following conditions are written to follow Thermo-Fisher's LabBooks software package.
Analytes: As (arsenic) and In (indium)

7.1 Acquisition Parameters

Identifier	Dwell time (s)	Channels	Spacing (u)	Measurement mode	Resolution
75As (KED)	0.05	1	0.1	KED	Normal
115In (KED)	0.05	1	0.1	KED	Normal
				# sweeps = 10	

7.2 Monitor Analytes

	Uptake	Wash
Minimum	30	30
Maximum	300	300

7.3 Survey Scan Settings

Start mass (u)	End mass (u)	Dwell Time (s)	Spacing (u)	Resolution	Measurement mode
4.60	245.00	0.01	0.2	Normal	KED

7.4 Interference Correction

Not applicable

7.5 Standards

Covered in calibration/control section

7.6 Quantification

Analyte	Measurement mode	Quantify	Internal Standard	Fit type	Weighting	Forcing	Use for Semi-Quant

75As (KED)	KED	Yes	115In (KED)	Linear	None	Blank	Yes
115In (KED)	KED	No	Use as Internal Standard	Linear	None	Blank	Yes
IS Recovery		Low warning limit: 80%			Low failure limit: 75%		
		High warning limit: 120%			High failure limit: 125%		

7.7 Ratios

Not applicable

7.8 Quality Control

7.8.1 Calibration Tests / Laboratory Control Standard (LCS)

Analyte	Low failure limit	Low warning limit	High warning limit	High failure limit
75As (KED)	80%	81%	119%	120%

7.8.2 Continuous Tests / Relative Stability Verification (RSV)

Analyte	Verify	Ignore concentration below	unit	Concentration warning limit	Concentration failure limit
75As (KED)	concentration	11	ppb	10%	15%

7.9 Autosampler

Time Settings:	Wash Time (s):	120	Take up Time (s):	45
Rack Settings:	Rack 1 Type:	60-vials (12x5)	Rack 2 Type:	60-vials (12x5)
Autotune Settings:	Autotune rack:	Standard	Autotune vial:	1
Rinse settings	Rinse Rack:	Rinse Station		

8 DATA ANALYSIS

8.1 Decision Criteria

8.1.1 Batch Acceptance Criteria

Arsenic should not be detected in the Negative Control Urine specimen above a level of 10 µg/L.

Arsenic should not be detected in the Negative Control Hair specimen above a level of 1 ng/mg.

Arsenic in the Positive Control Urine and Positive Control Hair specimens should quantitate within ±20% of the target value. See TOX-101 for more information.

8.1.2 Sample Acceptance Criteria

The Indium (Internal Standard) response in the unknown specimen(s) should fall within 80% and 120% of the Internal Standard response for the initial blank in the run.

8.2 Calculations

Quantitation is performed by constructing a multi-point calibration curve based on the ratio of the intensity for each calibrator level and the internal standard. The curve is forced through a blank urine specimen with no weighting. See TOX-101 for acceptable practices in calculating quantitative results.

For hair specimens, 5 mg of hair is used in the place of 0.1 mL of urine. Therefore, results as received from the instrument for hair should be divided by 50. For example, if a result of 100 µg/L of As is obtained for a hair specimen, that corresponds to 2 ng As per mg of hair.

When a hair specimen contains arsenic above the method's lower limit of quantitation, it may be analyzed again using a method of standard addition to verify the As concentration.

9 REPORTING

9.1 Measurement Uncertainty

Refer to CHEM-100, TOX-101 for guidance.

9.2 Reporting Limits

Interpretation of total urinary arsenic levels depends upon the specific case scenario presented. Generally, urine arsenic levels less than 50 µg/L are not of significance when considering an acute exposure scenario. For chronic exposures, urine arsenic levels less than 100 µg/L are considered less significant. Occupational exposures to arsenic may increase the total urinary arsenic level significantly.

10 Corrective Measures

Refer to TOX-101 for guidance.

11 Performance Characteristics

11.1 LOD/LOQ/Linearity

Matrix	LOD/LOQ (administratively set)	Upper LOQ
Urine	10 µg/L	1000 µg/L
Hair	1 ng/mg	20 ng/mg

11.2 Bias/Precision

Urine:	@30 µg/L	@400 µg/L	@800 µg/L
% Bias	2.23	3.20	3.06
% Repeatability	3.06	1.63	2.06
%Intermediate Precision	5.44	2.66	3.07

Hair:	@ 1 ng/mg (50 µg/L)	@ 10 ng/mg (500 µg/L)
% Bias	9.29	11.92
% Repeatability	1.47	2.73
%Intermediate Precision	2.71	3.01

11.3 Carryover

No carryover was observed when a negative control urine specimen was analyzed immediately following a 1000 ug/L calibrator. No carryover was observed when a negative control hair specimen was analyzed immediately following a 10 ng/mg control.

12 Limitations

12.1 Interferences

Interferences for urine and hair: no endogenous material/matrices interfered with the analysis of arsenic. For urine, a mixture of nickel, beryllium, cerium, indium, lithium, barium, bismuth, cobalt, lead and uranium at a concentration of 1000 ug/L for each element was analyzed and found not to interfere with the analysis of arsenic.

12.2 Processed Sample Stability

For urine, sample stability was evaluated up to seven days after the initial analysis and remained within ± 20% with refrigerated storage. For hair, sample stability was evaluated up to 24 hours after the initial analysis and remained within ± 25% with refrigerated storage.

12.3 Speciation

This procedure does not discriminate between the nontoxic organic forms of arsenic and the toxic inorganic form of arsenic.

13 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 Revision History

Revision	Issued	Changes
01	02/11/2022	Document reformat. Minor wording updates. 9.2 – added reporting limit for urinary arsenic 12.3 –moved speciation statement from introduction